

# The first genetic assessment of wild and farmed ball pythons (Reptilia, Serpentes, Pythonidae) in southern Togo

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## Abstract

The ball python (*Python regius*) is the world's most commonly traded python species for the “exotic” pet industry. The majority of these live snakes are produced via a number of python farms in West Africa that have been in operation since the 1960s and involved with “ranching” operations since the 1990s. However, to date no thorough taxonomic review or genetic studies have been conducted within its range, despite the fact that the evaluation of a species' genetic variability is generally considered mandatory for effective management. We used mtDNA sequence data and eight polymorphic microsatellite markers to assess the underlying population genetic structure and to test the potential of the nuclear markers to assign farm individuals to wild reference populations in southern Togo. Despite the relatively large distances between sample locations, no significant genetic population structure was found, either in mtDNA sequence data or in the microsatellite data. Instead, our data indicate considerable gene flow among the locations. The

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absence of a distinct population subdivision may have resulted from an anthropogenic driven admixture of populations associated with commercial wildlife trade activity in recent decades. Given the ongoing largely unregulated nature of the commercial ranching of ball pythons in West Africa, should a wild release component continue, as a first measure we recommend that the Management Authorities should develop an action plan with specific release protocols for python farms to minimise any potential negative conservation impacts resulting from admixture (genetic pollution) between farmed and wild individuals.

### Keywords

COI gene, microsatellites, population assignment, *Python regius*, West Africa, wildlife trade management

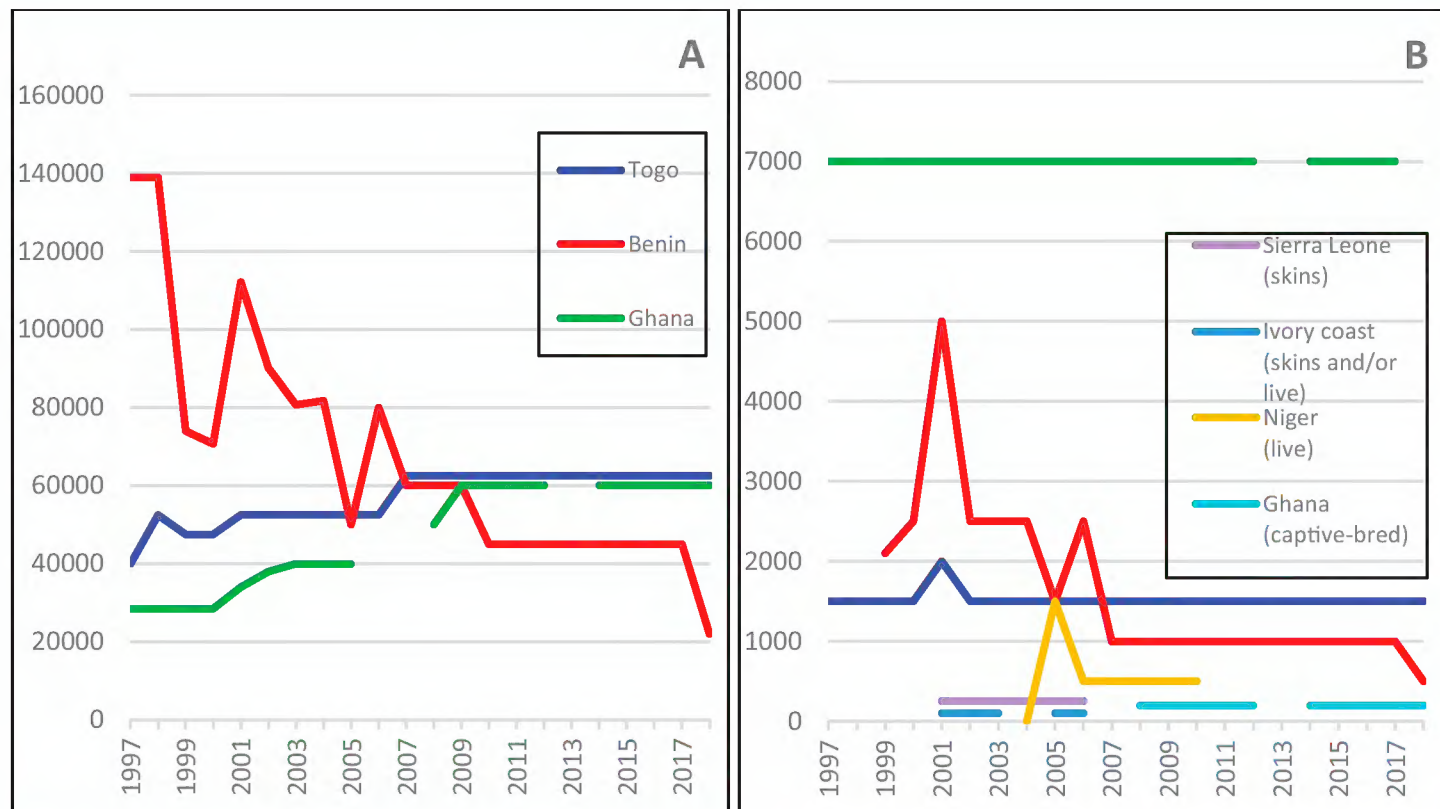
## Introduction

The ball python (*Python regius*) is native to open woodlands and savannahs of western Africa south of the Sahara extending east into north-western Uganda, and has been recorded from at least 18 countries (Barker and Barker 2006; Uetz et al. 2019). In 2009, the species was last assessed as “Least Concern” in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species with an “Unknown” population status (Auliya and Schmitz 2010). All Pythonidae species were included on CITES Appendix II in 1977 (except *Python molurus* that has been included on Appendix I since 1975) (UNEP 2019). The ball python is the world’s most common species of python traded internationally for the commercial exotic pet trade (Barker and Barker 2006; D’Cruze et al., in prep.).

Since 1997, several West African States within the range of ball python have established quotas for export, the majority of which relate to live specimens intended for commercial purposes (Fig. 1). In recent years the largest volumes were exported as “ranché” specimens (CITES Source Code “R”). For example, between 1997 and 2018, 3,628,030 live specimens were reported from the main exporting countries combined (Togo,  $n = 1,252,500$ ; Benin,  $n = 1,479,530$ ; Ghana,  $n = 867,500$ ) (CITES Trade Database, <https://trade.cites.org>). In the same period the total export quota for wild sourced live specimens was 206,101 (Togo,  $n = 33,501$ ; Benin,  $n = 32,600$ ; Ghana,  $n = 140,000$ ) (UNEP 2019). Between 2008 and 2018, Ghana established an annual export quota of 200 captive-bred specimens, except 2013; export quotas for captive-bred specimens were not established in Togo and Benin during the study period 1997–2018. Export quotas set by other West African nations are relatively negligible in terms of volumes (UNEP 2019; Fig. 1).

In recent decades, there has been a shift away from sourcing wild caught ball pythons and towards sourcing them through “ranching” initiatives via python “farms” in the three main exporting countries (Robinson et al. 2015), to the extent that in the last 10 years 95% of live exports from Togo were recorded using CITES source code “R” with the majority destined for the USA (CITES Trade Database, <https://trade.cites.org>; D’Cruze et al., in prep.). According to CITES, ranching is defined as the *rearing in a controlled environment of animals taken as eggs or juveniles from the wild, where they would otherwise have had a very low probability of surviving to adulthood* (<https://cites.org/eng/res/11/11-16R15.php>). A common part of the ranching system is the release





**Figure 1. A** Export quotas for ranched live ball python specimens from the three major exporting countries **B** export quotas for (1) wild live specimens from the three major exporting countries (for colours see **A**), for (2) wild specimens (live and skins), and for (3) captive-bred live specimens. Sources: <https://www.cites.org/eng/resources/quotas/index.php>, UNEP 2019.

of a percentage rate of neonates produced in captivity. In Togo for example, as recommended by Ineich (2006) this amount has been suggested to comprise 20% of the live snakes exported from Togo annually.

Between 1995 and 2016, numerous European Union (EU) and CITES-commissioned assessments and scientific studies were conducted in West Africa, including Togo (Segniagbeto et al. 2011). These initiatives aimed to evaluate management practices in the live reptile trade and in many cases had a particular focus on ball pythons (e.g., de Buffrénil 1995; Gorzula et al. 1997; Jenkins 1998; Affo 2001; Harris 2002; Harwood 2003; Ineich 2006; UNEP-WCMC 2010, 2014; Segniagbeto 2016). Although Ineich (2006) reported that trade was broadly being carried out under “relatively healthy conditions”, historically the results of these assessments have repeatedly reported a number of specific inconsistencies of the ranching strategies for Benin and raised questions for Togo that warrant additional scientific scrutiny (Ineich 2004, 2006; Toudonou 2003, 2004, 2007; Segniagbeto et al. 2011).

More recently, many of these inconsistencies have been confirmed as ongoing and have been summarised by D’Cruze et al. (in prep.) following a questionnaire-based study focused on ball python hunters in Togo. Specifically, this study found that: (1) the majority of surveyed hunters were not aware of national quotas relating to this CITES listed species; (2) that their harvest activity also involved illegal cross border collection and trade activities in other nearby range states (i.e., Benin, Ghana and Nigeria); and (3) that the release of gravid females collected in the wild and a proportion (20%, see above) of the resulting neonates (as part of the ranching program) was not monitored appropriately, with snakes being released without full care and con-



sideration given to key aspects, such as source location and the habitat of release sites (D’Cruze et al., in prep.).

Perceived and/or real inconsistencies relating to specific trade activities (as reported for ball python ranching – see above) can be forensically investigated by applying genetic methods that identify geographic origins and population structures of target species (Ogden 2012) (i.e., how is ball python ranching operated *per se*?). In addition, new information regarding the potential associated impacts of such trade activity on wild populations can also be elucidated (i.e., ball python conservation). However, to date no thorough taxonomic review or genetic studies have been conducted for the ball python within its range, despite the fact that the evaluation of a species’ genetic variability is considered mandatory for a fully effective and comprehensive management strategy (e.g., Sarre and Georges 2009; Frankham et al. 2014, and citations therein).

Here, we present the first molecular genetic analyses focused on the genetic structure and diversity of the natural population(s) of ball pythons in southern Togo [a region where the “vast majority of exploitation (in regard to ball pythons)” has been reported to take place (Harris 2002)] using eight polymorphic nuclear microsatellite loci and a fragment of the mitochondrial (mt) cytochrome c oxidase subunit 1 (COI) gene. COI is the most utilised marker for barcoding species (McGraw et al. 2012) and commonly used in phylogenetic studies on vertebrates (e.g., Saladin et al. 2019). Microsatellite markers are codominant, highly polymorphic and have high mutation rates and can therefore provide insight into small-scale genetic variation and ideally allow for the identification of specimens and their populations of origin (Schwartz et al. 2007; Palsbøll et al. 2013). Although microsatellites cannot directly impact a population negatively or positively, they have proven to be reliable indicators for the genetic diversity and allow for the comparison of genetic characteristics between populations, e.g. heterozygosity, allelic diversity, which has been noted to indicate levels of inbreeding or adaptive potential (Jump et al. 2009; Fraser et al. 2018; Lawrence et al. 2019). We used the microsatellite loci to test their predictive power for determining the origin of farmed (and traded) individuals and to assess the genetic differentiation between populations.

The main goal of this work is: (1) explore whether the genetic structure and divergence of wild ball python populations in Togo is consistent with their naturally expected low gene flow given their assumed low dispersal capacity (see below); (2) explore the role that commercial trade activities may have played to gain information on whether, and if so to what extent, ranching activities operating from facilities in python “farms” in Togo are impacting on wild populations; and (3) provide recommendations that can help inform existing and future initiatives focused on the conservation of this species.

## Methods

Currently there are nine known farms that are registered to conduct commercial captive breeding and ranching of reptiles in Lomé. Seven of these farms were visited dur-



ing this study. These seven farms are thought to be responsible for exporting the majority (> 90%) of specimens globally (Segniagbeto 2016).

### **Sample collection**

A non-invasive buccal swab method was applied to collect samples from 62 ball python specimens in Togo, including 21 samples from five of the nine known python farms currently located in Lomé and 41 samples from 12 wild populations located outside Lomé (Fig. 2). The number of samples differed between python farms and between locations of wild populations (ranging from 1–11) (Fig. 3, Suppl. material 1: Tables S1, S3).

### **DNA Extraction**

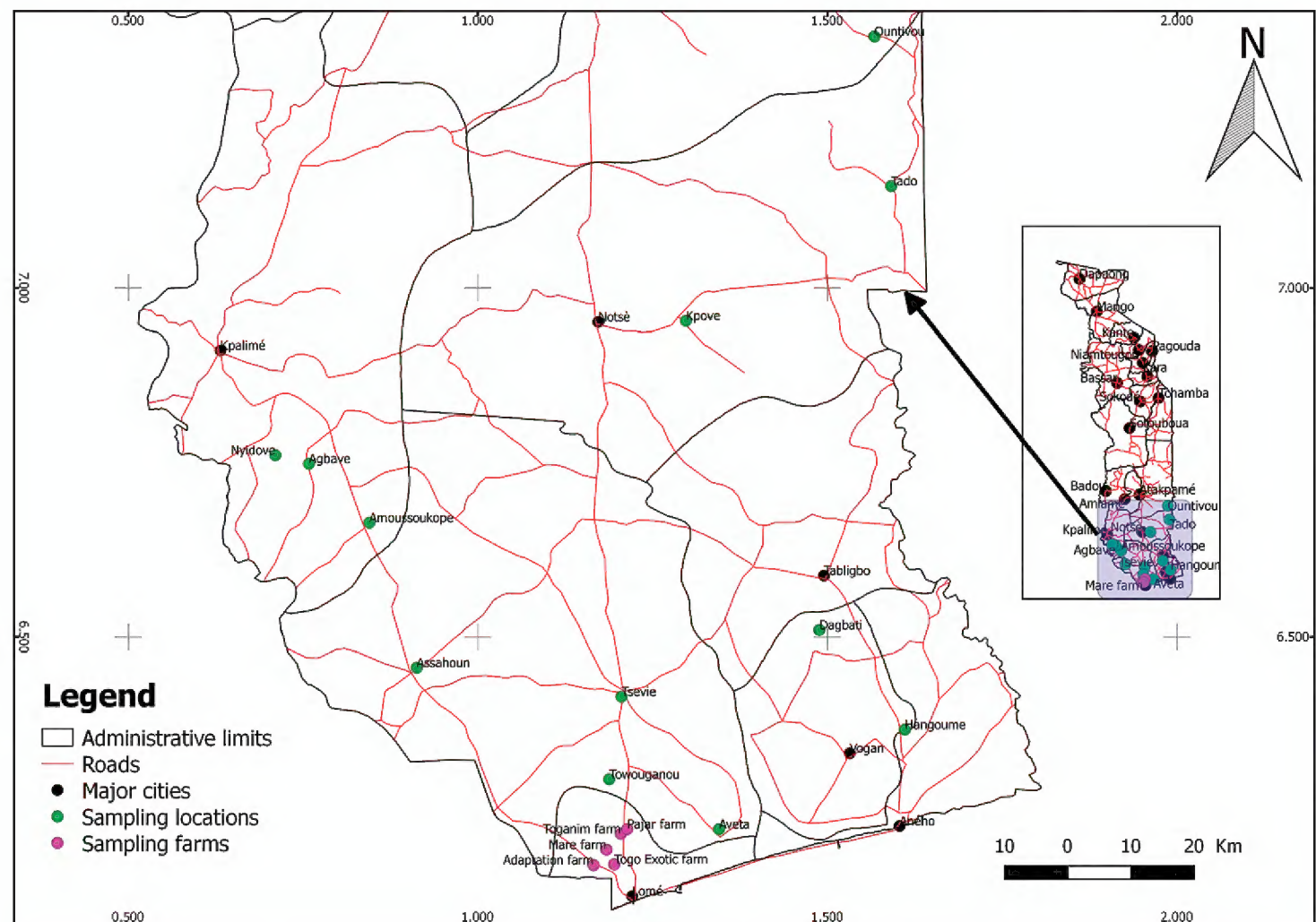
Total genomic DNA was extracted from swab samples using the Blood and Tissue kit by Qiagen (Hilden, Germany) following the manufacturer's protocol. DNA extracts are available from the ZFMK Biobank, Bonn. The COI segment (674 bp) with primers HCO2198-JJ and LCO1490-JJ (Astrin and Stüben 2008) was amplified via the polymerase chain reaction (PCR) in reaction volumes of 20 µl, including 2.5 µl of undiluted DNA template, and using the Multiplex PCR Master Mix (Qiagen). Thermal cycling was performed on GeneAmp PCR System 2700 instruments (Life Technologies, Carlsbad, USA) as follows: hot start Taq activation: 15 min at 95 °C; first cycle set, touch down (15 repeats): 35 s denaturation at 94 °C, 90 s annealing at 55 °C (–1 °C/cycle) and 90 s extension at 72 °C. Second cycle set (25 repeats): 35 s denaturation at 94 °C, 90 s annealing at 40 °C and 90 s extension at 72 °C; final elongation 10 min at 72 °C. Amplicons were purified with the ExoSAP-IT Purification Kit (USB Corporation, Cleveland, Ohio) and sequenced in both directions using the PCR primers at MacroGen Europe's commercial Sanger sequencing service (Amsterdam, NL). All newly found haplotypes were deposited in GenBank (accession numbers: Suppl. material 1: Table S1).

### **Mitochondrial sequence data analysis**

Molecular data were first phylogenetically analysed based on mtDNA to gain insights into spatial pattern of genetic variation and the level of genetic divergence among populations of the species in Togo.

The number of haplotypes was calculated using DnaSP 6 (Rozas et al. 2017). A median-joining (Bandelt et al. 1999) and a median (Bandelt et al. 1995) haplotype network was constructed in PopART v. 1.7 (Leigh and Bryant 2015) and SplitsTree v. 4.14.8 (Huson and Bryant 2006), respectively, to visualise relationships among haplotypes of the 12 localities (hereafter referred to as “populations”). Haplotype frequency distribution was mapped using ArcGIS 10.3.1 (ESRI, Redlands, CA, U.S.A.).



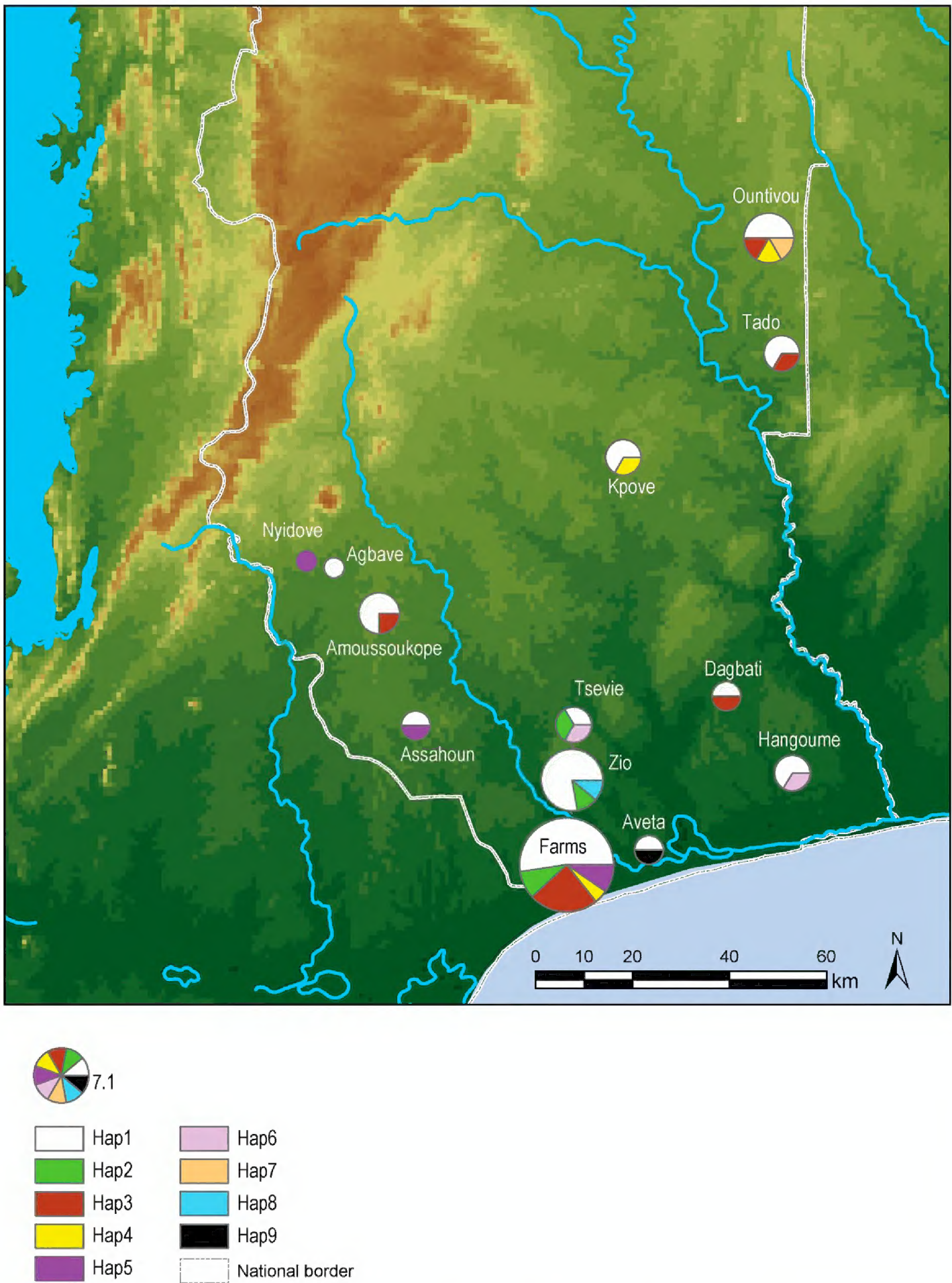


**Figure 2.** Southern Togo with sampling locations for wild *Python regius* (green) and housed in farms (red). Five specimens were sampled at each of three python farms, and at two farms each an additional three specimens were sampled. Number of wild specimens sampled per location: Hangoume,  $n = 3$ ; Dagbati,  $n = 2$ ; Kpove,  $n = 3$ ; Assahoun,  $n = 2$ ; Tado,  $n = 3$ ; Tsevié,  $n = 3$ ; Agbave,  $n = 1$ ; Nyidove,  $n = 1$ ; Amoussoukope,  $n = 4$ ; Ountivou,  $n = 6$ ; Towouganou (Zio),  $n = 11$ ; Aveta,  $n = 2$ .

We also tested for significant differences in nucleotide and haplotype diversity between sample locations using permutation tests implemented in the R script *genetic\_diversity\_diffs* v1.0.6 (Alexander et al. 2016; R Core Team 2019) with 10,000 iterations. The script resamples from the combined haplotype frequencies over all populations, in order to test whether the observed differences in haplotype diversity between specific populations, are greater than expected by chance. For this analysis, we grouped all farms together due to their small geographic distances, as we did with the neighbouring localities Agbave and Nyidove from which only one sample was available each.

Phylogenies were reconstructed by Maximum Likelihood (ML) and Bayesian Inference (BI) methods, using RAxML v. 8.2.10 (Stamatakis 2014) as well as MrBayes v. 3.2.7a (Ronquist et al. 2012). We used *Xenopeltis unicolor* (NCBI accession no. [LC075326](#)) as outgroup and included additional COI-sequences of *P. regius* available from GenBank ([AB177878](#); [KX012740](#); [KX012757](#); [KX012789–KX012791](#); [NC007399](#)). Optimal partitioning schemes and substitution models for each partition were selected using the Bayesian Information Criterion in a greedy search algorithm and linked branch lengths in PartitionFinder v. 1.1.1 (Lanfear et al. 2012, 2014). BI analyses were then run using models and codon partitions as selected by PartitionFinder.





**Figure 3.** Map of the spatial distribution of haplotypes of *Python regius* in Togo. Circle sizes correspond to haplotype frequency. Source of the map: <https://www.esri.com>.

ML tree construction was performed under the GTR-GAMMA model applying the codon partition scheme preferred by PartitionFinder. Nodal support of the tree was assessed by rapid bootstrapping algorithm with 1,000 bootstrap replicates.



## Microsatellite amplification, genotyping and analysis

We initially tested the cross-amplification of microsatellite loci previously described by Jordan et al. (2002) and Shaney et al. (2016). Out of these microsatellites, 11 loci cross-amplified in our samples and were genotyped for each sample using fluorescently labelled primers (for accession numbers see Table 1). Some of the primers were modified in their length to adjust the annealing temperature for a multiplex reaction. Before multiplexing, all primer pairs were tested in singleplex PCRs to check for correct amplification of the desired fragments. PCRs were performed in a 12,5 µl reaction mix containing 5–40 ng DNA, 200 µM dNTP's, 0,5 µM each primer pair (reverse primer of each pair was labelled, see Table 1), 5× Q5 reaction buffer and 1 U Q5 High-Fidelity polymerase (New England Biolabs). Amplification conditions were as follows: 98 °C for 30 s followed by 33 cycles at 98 °C for 10 s, 64 °C annealing temperature for 30 s and 72 °C for 30 s, with a final extension at 72 °C for 2 min. Amplification products were separated on an ABI 3730 automatic sequencer. Raw data were analysed and evaluated using GeneMapper ID v3.2.1 (Applied Biosystems).

## Microsatellite analysis

All markers were checked for scoring errors, large allele dropout and the possible presence of null alleles using Micro-checker v2.2.3 (van Oosterhout et al. 2004). Departure from Hardy–Weinberg equilibrium (HWE) was tested for each locus by Fisher's exact tests (Guo and Thompson 1992) using a Markov chain Monte Carlo (MCMC) approach with 10,000 steps and 1,000 iterations as implemented in GenePop v. 4.2 (Raymond and Rousset 1995). GenePop was also used to test linkage disequilibrium (LD) for all pairs of loci. P-values of LD were corrected using the FDR-method (Benjamini and Hochberg 1995). The number of alleles, polymorphic information content (PIC) (Botstein et al. 1980), expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosity were calculated per locus using Cervus v. 3.0.7 (Marshall et al. 1998; Kalinowski et al. 2007). FSTAT (Goudet 1995) was used to estimate and test (with 10,000 permutations)  $F_{ST}$  values between wild populations as well as between wild populations and farm individuals. GenAlEx (Peakall and Smouse 2006, 2012) was used to compute the probability of identity (PI) with increasing locus combinations, i.e., the probability that two randomly chosen individuals in a population have identical genotypes.

Isolation by distance (IBD) was examined for the “wild” populations with reduced major axis (RMA) regression and Mantel test on matrices of genetic and geographic distances using IBD v.1.52 (Bohonak 2002) and 10,000 randomisations. Pairwise geographic distances between localities were calculated with the Geographic Distance Matrix Generator (Ersts 2019) and log-transformed. Genetic distance matrix ( $F_{ST}/[1-F_{ST}]$ ; Rousset 1997) was obtained with GenePop, based on the eight polymorphic loci.

Individual assignment tests were performed to assign farm individuals to the population they have the highest probability of belonging to using a Bayesian approach



**Table 1.** Microsatellite primers used for multiplex reaction.

Sequence name	5' Modification	Primer Sequence (5'-3')	Genbank Accession No.
MS27-F	6-Fam	TTACACAACAACCGCCATAG	AF403219
MS27-R_mod*		TCCTTCTTATCCTGTTTACTCTGT	
KE959105.1-F	6-Fam	CACTGTTTTTGGGCCATCTCC	KE959105
KE959105.1-R		GGGTTTAGGATGTGTTCTGATTCC	
KE955519.1-F_mod*	6-Fam	ATTTTAGCTGCAGGCTGTGG	KE955519
KE955519.1-R		TCTGCTAGGGGCAAAACTGGG	
KE961431.1-F	6-Fam	GAAGGGAGGCCCAAATATCC	KE961431
KE961431.1-R		GAGAGACCTGGTGCAAACCC	
KE961083.1-F_mod*	6-Fam	GTCCCAAACATCCAGAGGG	KE961083
KE961083.1-R		GGATCAAACCTGGACAAGCC	
KE955203.1-F	Joe	TGCATTTTCTCTTCCACAGGG	KE955203
KE955203.1-R		ATCTTCTGGGGAACCAACCC	
MS16-F	Joe	GAGTTCTGGTCTTGCTTTCG	AF403208
MS16-R		CAGGTACAACCTTCTCCAAC	
KE966557.1-F	Joe	GCCTCCTACTCAAAGGGTGG	KE966557
KE966557.1-R		CATGGGAGGCAAGGTAAAGG	
MS9-F	Tamra	CAGTGGGCTTGAGATTGAC	AF403201
MS9-R_mod*		CCATTCCTTAAAACACTCTCACTC	
MS13-F	Tamra	AACAGAGAAGCACAATCACC	AF403205
MS13-R_mod*		TGGCTCTCACTTGATATATTAGAAG	
MS5-F	Tamra	TAGGGTGTTCAGTCATTGCTC	AF403197
MS5-R		TGGCATCCAGCAGTCATAG	

\* modified primer length

according to Rannala and Mountain (1997) implemented in the program GeneClass2 (Piry et al. 2004). This method has been shown to outperform other assignment techniques, and has been specifically demonstrated to obtain high assignment accuracy for smaller sample sizes (Cornuet et al. 1999). Moreover, this assignment method has the advantage that it does not assume that the true population of origin is among the sampled populations (Manel et al. 2002). We included the eight polymorphic loci only, assumed a threshold of 0.05 and applied a Monte Carlo resampling of 100,000 simulated individuals (Paetkau et al. 2004). For broad comparison, we also performed self-assignment tests for the individuals from wild populations using the same parameter setting. We considered individuals with an assignment score > 0.7 to be assigned with confidence (Dellicour et al. 2011).

Results

MtDNA sequence information and phylogeny

The extraction of DNA from buccal swabs of 60 individual specimens from Togo proved successful; for two samples DNA extraction failed. The aligned sequence data set of these samples contained 674 bp with 13 variable characters of which 10 were parsimony informative. Translation of the gene segment revealed no frameshift muta-



tions or premature stop codons. Both BI and ML trees show that samples are split into three major clades, which, however, show no geographic structure and are only weakly differentiated (Suppl. material 1: Fig. S1). These clades are consistently recovered in the median network (Suppl. material 1: Fig. S2). Sequence comparison revealed a total of nine haplotypes, including a haplotype found by Dong and Kumazawa (2005) (AB177878); the nine haplotypes were deposited in GenBank (MN295674–82). One haplotype (hap1) was shared by all localities except Nyidove (close to the Ghanaian border); three haplotypes (hap7, hap8 and hap9) were unique to their geographic population (Ountivou, near the Benin border; Towouganou [Zio district] and Aveta), and not represented among the samples from any farm (Fig. 3). Remaining haplotypes (which differed from the most common haplotype by only 1–4 mutations) were found at moderate to low frequency (Figs 3, 4). Five among nine haplotypes (based on 21 individuals) were represented among farm animals.

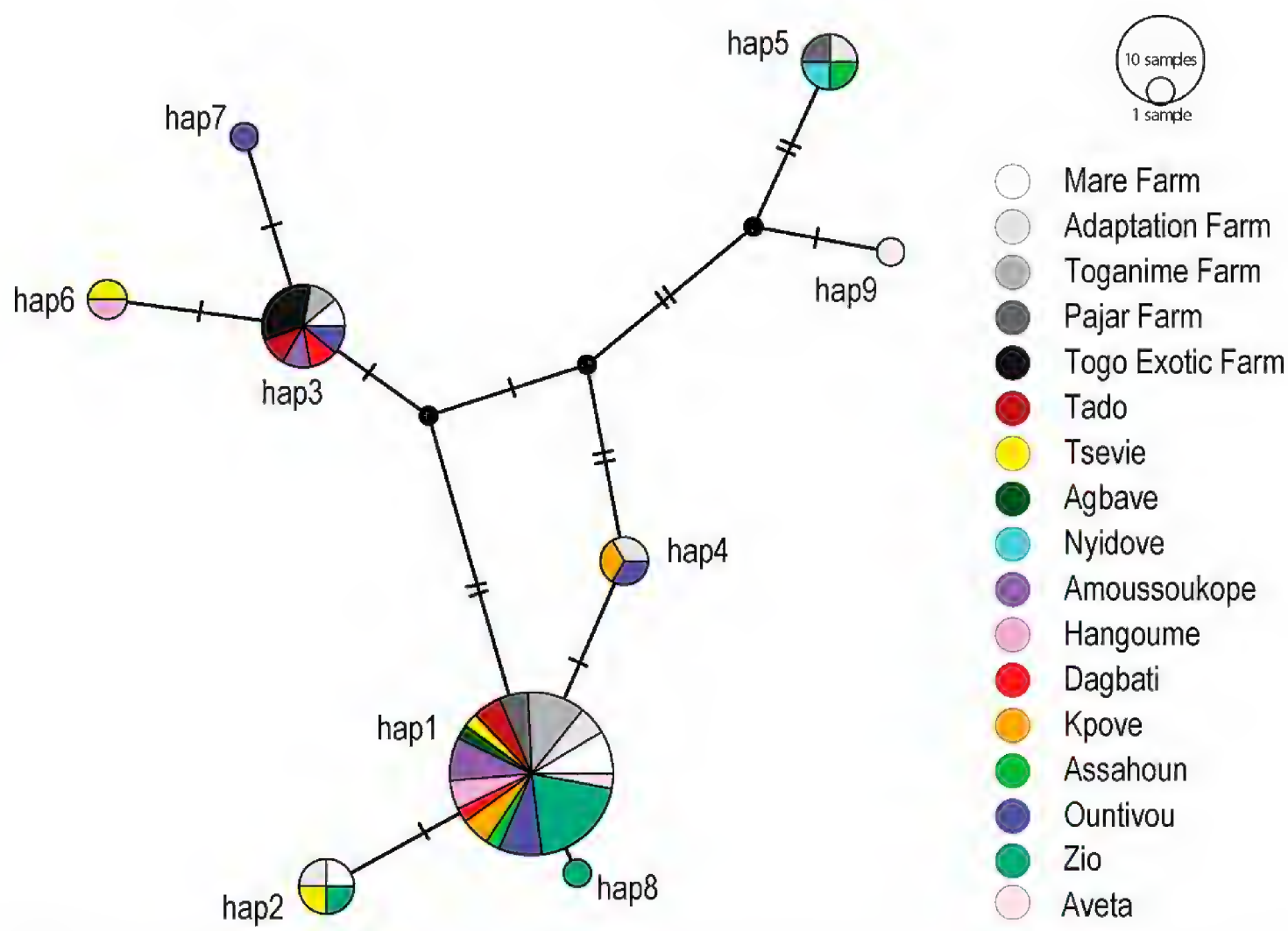
Consistent with the phylogenetic trees, the haplotype network does not inform on a specific geographic structure and pairwise comparison of genetic diversity between the localities revealed no significant differences (Fig. 4, Suppl. material 1: Table S2).

### Microsatellites – Genetic diversity, and individual assignment

Seven out of 60 individual DNA samples could not be amplified for any of the microsatellite loci and were excluded from subsequent analyses. At least eight microsatellites were polymorphic and four of them showed higher polymorphism with PIC values  $> 0.7$  (Table 2). None of the loci showed indications for the presence of null alleles, large allele dropout or stuttering. The sample set from the “Adaptation farm” showed a higher number of homozygotes at locus 5-MS16. However, because these farm samples may originate from different populations, we considered this excess as uncritical for subsequent analyses. Tests for LD between all pairs of loci across populations yielded no significant genotypic disequilibrium; none of the loci deviated significantly from HWE. For polymorphic loci, the mean number of alleles was seven (2–25 alleles per locus), the observed heterozygosity ranged from 0.02 to 0.91, and the expected heterozygosity from 0.01 to 0.93. The probability of two random animals having identical genotypes was estimated at  $2.0 \times 10^{-8}$  (Table 2).

Information on population differentiation can ideally serve the management of genetic populations or, in our case, monitor national management regimes. The level of genetic differentiation ( $F_{ST}$ ) between localities where wild individuals were sampled and specimens sampled from farms varied from 0 to 0.09 (Suppl. material 1: Table S3); none of the pairwise  $F_{ST}$ -values was significant, and there was no evidence of isolation by distance across populations ( $Z = -11588.18$ ,  $r = 0.24$ ,  $p \leq 0.08$ ). Notably,  $F_{ST}$ -values among pairs that included a farm were remarkably low, indicating a potentially higher level of individuals from different populations among the farm samples that minimise the degree of genetic differentiation between farms and wild populations.





**Figure 4.** Haplotype network based on 674 bp of COI gene from 60 specimens of *Python regius* from Togo. Each circle represents a haplotype with its size proportional to the frequency of the haplotype. Ticks on branches connecting the haplotypes indicate nucleotide mutations. Localities are indicated by different colour.

**Table 2.** Genetic variability at 11 microsatellites applied in *Python regius*. Ho = observed heterozygosity; He = expected heterozygosity; PIC = polymorphism information content; PI = probability of identity for increasing locus combinations.

Locus	Repeat motif	Allelic range	No. of alleles	Mean no. of alleles	Ho	He	Missing data (%)	PIC	PI
12-MS27	(TCTC)7	0	1	1	–	–	0	–	1.0
3-KE959105	(AATC)8	0	1	1	–	–	0	–	1.0
1-KE955519	(AC)90	14	8	3	0.72	0.72	0	0.68	1.2e <sup>-1</sup>
14-KE961431	(TC)10	0	1	1	–	–	0	–	1.2e <sup>-1</sup>
2-KE961083	(TTCC)13	22	12	5	0.85	0.87	3.64	0.85	3.8e <sup>-3</sup>
7-KE955203	(TTC)35	35	2	1	0.02	0.02	0	0.02	3.6e <sup>-3</sup>
5-MS16	(AAAG)12	30	10	4	0.75	0.82	5.56	0.78	2.3e <sup>-4</sup>
6-KE966557	(AC)15	4	3	1	0.09	0.09	1.82	0.09	1.9e <sup>-4</sup>
8-MS9	(AAAG)18	24	7	4	0.78	0.79	0	0.75	1.5e <sup>-5</sup>
17-MS13	(TTTC)16	16	5	3	0.76	0.74	0	0.68	1.9e <sup>-6</sup>
9-MS5	(TTTC)17	94	25	5	0.91	0.93	0	0.92	2.0e <sup>-8</sup>
Mean			7	3		0.45		0.43	



Self-assignment of individuals from wild populations was successful and correct in 94% of the samples, with assignment probabilities ranging between 0.66 and 0.96 (mean 0.86); only two of these correctly allocated samples were assigned with lower confidence (0.66 – 0.70); (Suppl. material 1: Table S4). Sample MA26 (from Tsevié) was assigned with a slightly higher confidence to a different population than to the one where it was sampled from; sample MA50 (from Zio) couldn't be assigned to any of the wild population. In “real life” assignment, two of the farm individuals (MA15, MA19) could be assigned with high confidence to one of the wild reference populations, while a further five samples (MA3, MA11, MA12, MA15, MA20) were assigned with a probability > 0.6 (Suppl. material 1: Table S5).

## Discussion

Severe inconsistencies in trade activities relating to this species have been previously reported from the main exporting countries in West Africa (Benin, Togo and Ghana) (see Ineich 2004, 2006; Toudonou 2003, 2004, 2007), yet despite the recognised importance of such data for sustainable management strategies, genetic variation among the species' populations has never been assessed, and molecular forensic methods for monitoring purposes have not been developed so far.

As such, our study is the first to report on the regional molecular phylogeny and genetic population differentiation of ball pythons from West Africa and to test the suitability of polymorphic microsatellites for tracking the origin of farmed individuals. Overall, these initial genetic findings from Togo indicate a relatively high mixing rate of ball pythons at the sampled localities, both within farms and wild populations, with no apparent bio-geographical trends, which may likely mirror the long-lasting anthropogenic use, and commercial trade of this species in Togo and other neighbouring range states in West Africa. However, further research to identify the degree of differentiation in non-harvested regions, and potential genetic homogenization at a larger spatial scale is required to verify this conclusively.

## Ball python population genetics in Togo

There are no significant geographical or climatic barriers in the sample area and gene flow between populations of ball pythons has likely occurred over the last 10,000 years. However, unlike our study, genetic studies focussed on wild populations of other savannah inhabitant reptile species in West Africa have revealed phylogenetically distinct clades [e.g., the African helmeted turtle (*Pelomedusa subrufa*) (Vargas-Ramírez et al. 2010; Wong et al. 2010), the Puff adder (*Bitis arietans*) (Barlow et al. 2013), and the Egyptian cobra (*Naja haje*) (Trape et al. 2009)]. Likewise, phylogenetically distinct clades have also been previously reported for ball python prey species [e.g., Misonne's soft-furred mouse (*Praomys misonnei*)] (Nicolas et al. 2011).



Furthermore, stronger population subdivisions than those observed would be expected, at least across a wider geographic scale, given the low assumed dispersal capacity of ball pythons. Although an important research priority, to date field research provides some information on habitat use (Luiselli and Akani 1999), while studies on movements and home range sizes of ball pythons in West Africa are almost lacking. Little published information is currently available on daily and seasonal activities (Luiselli et al. 2007), however based on field experience and discussions with collectors in Benin, the species likely exhibits narrow home range (Toudonou, pers. comms.). Overall, it is suggested that the ball python represents a rather sedentary species (Luiselli, pers. comm.) that likely reflects other congeneric sedentary species, such as the Short-tail python (*Python brongersmai*), of which specimens have been shown to cover a mean distance of less than 200 metres in ca. 50 days (Erdelen et al. 1997, in: Auliya 2006).

There are other factors that can influence snake movement and dispersal, such as the seasonal flooding of python habitat during the wet season (Auliya 2006). Activity levels can also vary between savannah and forest populations (Luiselli and Angelici 1998), and males of congeners have reported higher activity levels when searching for females during the breeding season (Slip and Shine 1988). Although such aspects may have certainly influenced ball python population genetics in Togo, it is important to note that this species is reported to select specific microhabitats that provide numerous shelter opportunities and a constant prey source (Barker and Barker 2006).

Similarly, with regards to the ball python specimens sampled within python farms, haplotype diversity indicates that python farm ranching activity has historically targeted several populations but does not provide a clear spatial pattern or “trade chain” in this regard. The extent to which this observed lack of well-defined haplogroups is the result of regional trade activity or continuous historical gene flow/long-distance dispersal (or indeed *vice versa*) cannot be determined based on the mtDNA sequence data alone. However, it does raise a number of important questions regarding the impact of ongoing commercial trade activity on remaining wild populations.

In particular, it is currently unclear whether any of the haplotypes identified in Togo during this study actually originated and / or have current core distributions in neighbouring range States. This is a distinct possibility given that researchers have reported illegal cross-border hunting of ball pythons (de Buffrénil 1995) and other reptile species, such as the Müller’s sand boa (*Eryx muelleri*) (Vignoli et al. 2015).

### **Potential impacts of commercial trade on ball python populations**

Our initial genetic findings line up with results obtained from a recent questionnaire-based study focused on python hunters in Togo, which reported that the collection and subsequent release of ball python ranched specimens was carried out in a relatively *ad hoc* and diffuse manner, without an effective monitoring process in place (D’Cruze et al., in prep.). Specifically, this study raised concerns that ball pythons may be released in insufficient numbers, in inappropriate habitats and geographic locations that may



be at least partly responsible for reported decreases in local wild ball python populations over the last five years (D’Cruze et al., in prep.).

It is beyond the scope of this study to assess the full impact that trade and associated ranching activity has had on the conservation status of wild ball python populations in Togo and other neighboring range states in West Africa. However, the challenges associated with implementing proper wild release protocols, and the multiple risks posed to focal species, their associated communities and ecosystem functions in both source and destination areas (including disease introduction and genetic pollution) are well known and should be mitigated (IUCN/SSC 2013).

In particular, ball pythons are known to harbour a number of infectious diseases (e.g., cryptosporidium, Yimming et al. 2016; nidoviruses, Uccellini et al. 2014) and parasites [including ticks (e.g., *Amblyomma latum*, Corn et al. 2011)]. For a recent review of the scientific literature (published between 2009 and 2019), which identified a list of nearly 150 underlying pathogens associated with this species, please see Green et al. (2020). The fact that ball pythons are being ranched under intensive commercial captive conditions prior to their release in large numbers (as has been reported in the CITES trade database), together with concerns regarding a lack of proper biosecurity measures at these facilities (D’Cruze et al., in prep.), arguably makes a higher level of monitoring and evaluation a key priority.

Moreover, the translocation of individuals of non-local origin may lead to introgression that disrupts spatial genetic structure, alters local genetic diversity, and ultimately threatens local adaptations (e.g., Söderquist et al. 2017 and references therein). Hybridisation between translocated farmed and wild individuals might also be a driver of biodiversity loss (Randi 2008; Sutherland et al. 2006; Söderquist et al. 2017).

### Limitations of the genetic study

Our study provides only an initial insight into ball python genetic diversity in Togo. Yet, regardless of the low sample size per population, more than 90% of the samples from wild captures of ball python were correctly assigned to their population of origin, while 10% of farm samples could be assigned with high probability to one of the wild populations (and ca. 1/3 with a probability > 0.6). This could indicate that farms harvest populations, which have not been sampled in our study. However, the relatively low percentage of individuals assigned is similar to those reported in other studies and the limited assignment accuracy may result mainly from population pairs with a  $F_{ST} < 0.05$  (Manel et al. 2002).

We also acknowledge that identifying the full population status and or exact geographic region of origin of ranched ball pythons in the wild requires a much denser sampling from a wider geographical area (including populations that have been subject to harvest in Ghana and Benin, in addition to reference samples of non-harvested populations across the species’ range), and most importantly, a higher sample size per (sub-) population (i.e., 25–30 samples; Kalinowski 2005; Hale et al. 2012). This would enable



us to identify geographic regions where substantial genetic admixture of farmed genotypes with wild populations may or may not have occurred. Moreover, when a larger sample size is applied, polymorphism may be revealed at the three additional loci (12-MS27, 3-KE959105, 14-KE961431) that were monomorphic in the samples used in this present study, which consequently may contribute “decisively” to forensic assignment.

It is important to note that larger than expected dispersal rates for this species may be partly responsible for the genetic admixtures reported in this study and more ecological studies are needed in this regard. However, based on current knowledge (cf. above), the ball python is likely a relatively sedentary species, assumed low dispersal capacity.

## Recommendations

The long-term sustainability of the large-scale ranching and subsequent export of ball pythons from the main exporters in West Africa, such as Togo, is undermined by a lack of data on the status of wild ball python populations including their distribution, population trends and genetic structure. Such data is essential to effectively manage, monitor and evaluate the impact that this type of commercial trade activity may have on the conservation of this species and is an urgent priority in this regard. We recommend that future studies looking to build on our findings should aim to reduce the geographic sampling gaps to provide a denser coverage of samples over a larger area (including non-harvested regions), include samples from neighbouring range states (especially Benin and Ghana), include a higher number of samples per population/locality (25–30), and an increased number of relevant nuclear polymorphic markers (e.g., SNPs, microsatellites or SNP-STRs), to help better assign individual ball pythons to specific populations. Thus, systematic monitoring across a larger scale is needed to gain more insight of the spatial genetic population structure and the processes that are potentially associated with the uncoordinated translocation of farmed and wild individuals.

However, such research initiatives can be difficult to implement, time consuming and costly to fund (D’Cruze et al. 2009). In the interim, we recommend that Management Authorities should urgently develop a national action plan and specific release protocols for python farms to minimise any negative conservation impacts resulting from genetic pollution (whilst also minimising any risk of disease introduction). Should such efforts prove unsuccessful or unviable, additional measures such as a reduction in the number of ball pythons that are both captured and released may be required as part of a more precautionary approach. This type of action is not unprecedented; a quota reduction has already been adopted by the neighbouring range state and third largest exporter Benin, which recently reduced its ball python export quota from 45,000 ranched specimens in 2017 to 22,000 in 2019 (UNEP 2019). Whatever steps are taken, it is important to draw attention to the fact that given the cross-border harvest activity that underpins the ball python trade in West Africa, any future initiatives should not be restricted to Togo only but also extend to Benin and Ghana too (Gorzula et al. 1997; Jenkins 1998).



## Conclusions

This study represents the first molecular genetic characterisation of ball pythons in Togo, one of the world's most traded snake species. Despite relatively large distances between sampled locations covering more than 12,773 km<sup>2</sup> (an estimate based on relevant district area sizes) no significant genetic population structure was identified, potentially implying a long-lasting human influence through domestic and international trade activities, or higher (long-distance) dispersal rates in ball pythons than the species' natural history would suggest. Although the ball python is not yet considered to be threatened by extinction, a modified genetic structure and a potentially associated loss of local adaptations should be nevertheless of concern from a conservation perspective. Self-assignments were correct for more than 90% of the samples from wild populations, and almost 1/3 of samples from farmed individuals could be allocated with higher probability to their potential population of origin. Although preliminary in nature, this study is the first of its kind for the ball python in West Africa. It has clearly demonstrated potential for the genetic assignment of ranched individuals that can assist management authorities with the ability to better monitor aspects of the ranching system and to trace trading activities in future.

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## **Supplementary material I**

### **Supplementary tables and figures**

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Data type: molecular data

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